

FLUORESCENCE AND UV-VIS SPECTRAL ANALYSIS
OF THE INTERACTION OF ADENOSINE DEAMINASE
AND COMPOUND D011_186

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Adenosine deaminase (ADA) is an enzyme important in mammalian purine metabolism and in maintenance of the immune system. Its activity increases in pathological conditions, resulting in decrease in the concentration of anti-inflammatory adenosine, with aggravation of inflammation. Therefore, the discovery and development of ADA inhibitors represent an actual biochemical and pharmacological task. We tested approximately 100 new synthesized aniline and piperazine derivatives for their ADA inhibitory activity. The present study describes the inhibition of ADA by one of the aniline derivatives tested, D011_186. The compound exhibited potent inhibitory activity with an IC_{50} value of $40 \pm 1.1 \mu g/mL$. The interaction between the ADA and D011_186 was investigated using UV-Vis and fluorescence spectroscopy techniques. Differential UV-Vis spectroscopy analysis of the ADA-compound interaction revealed no significant difference between the optical absorption of their mixture relative to the sum of their individual spectra. Next, 2D and 3D fluorescence spectroscopy methods were applied to assess ADA-compound interaction. The individual fluorescence spectra of ADA and compound were compared with the spectrum of their mixture, however, no changes in the recorded fluorescence spectra reflecting their interaction were detected. Thus, although the compound D011_186 inhibits ADA activity it is not accompanied by a change in the orbitals of their optical electrons.

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Keywords: adenosine deaminase (ADA), aniline derivative D011_186, IC_{50} , UV-Vis spectroscopy, excitation-emission matrix (3D EEM) spectroscopy.

Introduction. Adenosine deaminase (ADA) is a key enzyme in purine metabolism that plays a crucial role in the development and maintenance of the immune system. Its activity is elevated in various pathological conditions, including different cancers, infectious and autoimmune diseases [1]. Increased ADA activity leads to a reduction in the concentration of its substrate, adenosine [2, 3]. Since adenosine is known as anti-inflammatory agent, decreasing its level may contribute to the promotion of inflammatory processes [4]. Therefore, the discovery and development of new ADA inhibitors are important for the management of numerous pathological conditions. We tested approximately 100 compounds of aniline and piperazine derivatives for their effects on ADA activity [unpublished].

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Fluorescence spectroscopy in protein–ligand studies has advanced rapidly due to theoretical and technological progress [5, 6]. A wide range of fluorescence techniques, such as two-dimensional fluorescence (2D), steady-state and time-resolved fluorescence, synchronous fluorescence, and excitation–emission matrix (3D EEM) spectroscopy are now extensively employed to study biological processes and investigate protein–ligand interactions [7, 8]. 3D EEM spectroscopy, involves recording fluorescence intensities over a wide range of excitation and emission wavelengths. In 3D EEM, the excitation spectrum reflects how the emission intensity at a fixed wavelength changes as the excitation wavelength varies and vice versa [9]. This work is devoted to the study of the inhibition of ADA by a new synthesized aniline derivative D011_186, as well as the interaction of ADA with D011_186 using optical absorption and fluorescence spectrometry methods.

Materials and Methods. ADA purified from bovine lung as is described in [10] was used. The activity of ADA was assayed using the phenol-hypochlorite colorimetric method, described in [11]. The amount of ammonia liberated during the ADA-catalyzed deamination of adenosine was measured by monitoring the absorbance of the assay mixture at 630 nm. Double recrystallized ammonium sulfate was used as a standard and the enzyme activity was expressed in IU/mg protein.

Compound D011_186. D011_186 is an aniline derivative [12]. The chemical structure of D011_186 is shown in Fig. 1.

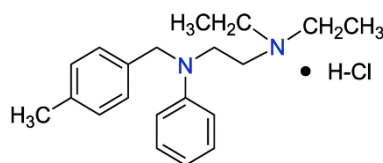


Fig. 1. Compound D011_186.

This compound is insoluble in water, but soluble in ethanol containing solvents. Since ethanol can affect ADA activity, its final concentration in the enzyme assay mixtures was maintained below 1.9%, which did not affect ADA activity in specific tests.

The Influence of D011_186 on ADA Activity. The IC_{50} value in ADA inhibition by D011_186 was calculated from the dependence of the enzyme activity from concentration of the compound, using computer program GraFit Version 5 (Leatherbarrow, 2001, Erithacus Software Ltd., Horley, U.K) as described in [13].

The preliminary prepared solution of the compound in 96% ethanol was diluted 10 times by bi-distilled water and used as stock solution. The appropriate aliquots of stock solution were added to the ADA assay mixtures.

Excitation–Emission Fluorescence Study of ADA Interaction with D011_186.

The intrinsic 3D EEM fluorescence of tryptophan residues in bovine ADA was measured by recording fluorescence spectra at excitation wavelengths ranging from 200 to 450 nm in 10 nm increments. The emission was collected over the 200–450 nm range as well. The obtained data were plotted and analysed using OriginPro 8.5.0 SR1 software.

Equipment. The UV-Vis absorption spectra were measured on Agilent Cary UV-Vis spectrophotometer, using quartz cuvettes with optical light path of 1 cm.

Fluorescence spectra were recorded using an Agilent Cary Eclipse fluorescence spectrometer in quartz cuvettes with a 1 cm optical light path.

Results and Discussion.

Calculation of Inhibition Parameters. The IC_{50} value of the aniline derivative D011_186 in inhibition of ADA activity was calculated using the dependence of the enzyme activity from the compound concentration in range of 0.63 nM to 1.77 nM (see the section methods). The obtained IC_{50} value (see Table) was used to calculate the K_i parameter applying the Cheng–Prusoff Equation (1).

$$K_i = \frac{IC_{50} \cdot K_m}{[S] + K_m}, \quad (1)$$

where $[S]$ is the concentration of substrate, used in the study (in our experiments the concentration of adenosine was 400 μM); K_m is the Michaelis constant for the catalyzed by ADA reaction of adenosine deamination (in our experiments it was 100 μM).

The obtained values of both inhibition parameters are presented in Table.

The IC_{50} and K_i values for anyline derivative D011_186 in inhibition of ADA

Compound	IC_{50} , $\mu g/mL$	K_i , μM
D011_186	40 ± 1.1	0.024

In Table, the low IC_{50} value indicates strong ADA-inhibitory potency of the compound DO_186, while the low K_i value suggests a high binding affinity between the compound and the enzyme.

These results demonstrate that compound D011_186 is an effective inhibitor of ADA.

UV-visible Absorption Spectral Analysis of Interaction of ADA and D011_186. The UVvisible absorption spectra of ADA and compound D011_186 are shown in Fig. 2.

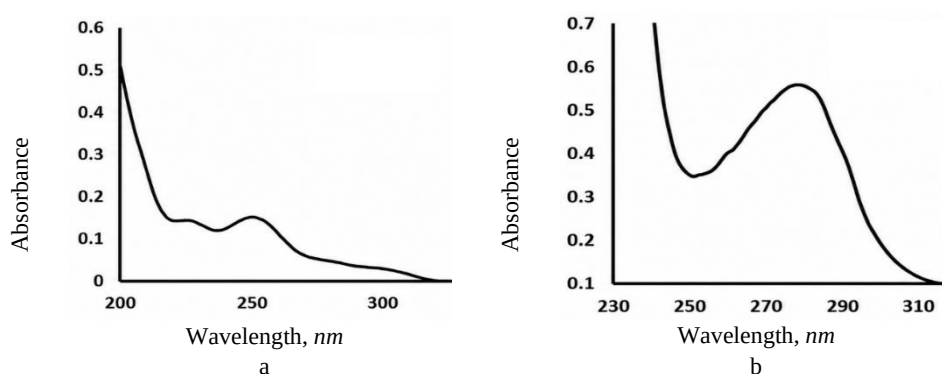


Fig. 2. The UV-Vis absorption spectra of 5.6 nM ADA (a) and 1.77 nM D011_186 (b) recorded using a quartz cuvette with an optical path length of 1 cm.

ADA (Fig. 2, a) displays a typical protein absorption profile with a high maximum around 220 nm (due to absorption of peptide bonds) and a band at 260–290 nm region (attributed to aromatic amino acid residues tryptophan, tyrosine and phenylalanine).

Compound D011_186 (Fig. 2, b) exhibits an essentially lower intensity absorption in the short-wavelength region and a band in the region of 240–260 nm. This behavior is characteristic of $\pi \rightarrow \pi^*$ electronic transitions associated with conjugated organic systems (Fig. 1).

Differential UV-Vis spectroscopic analysis of the ADA-compound interaction revealed no significant difference between the optical absorption of their mixture relative to the sum of their individual spectra.

Thereafter, fluorescence spectroscopy was employed to further investigate possible interaction-induced changes in the electronic spectra of the compounds.

Influence of the D011_186 on Tryptophan Fluorescence in ADA. 3D-EEM fluorescence spectra of ADA (Fig. 3, a), D011_186 (Fig. 4, a), and their mixture (Fig. 5, a) were recorded as described in section Materials and Methods.

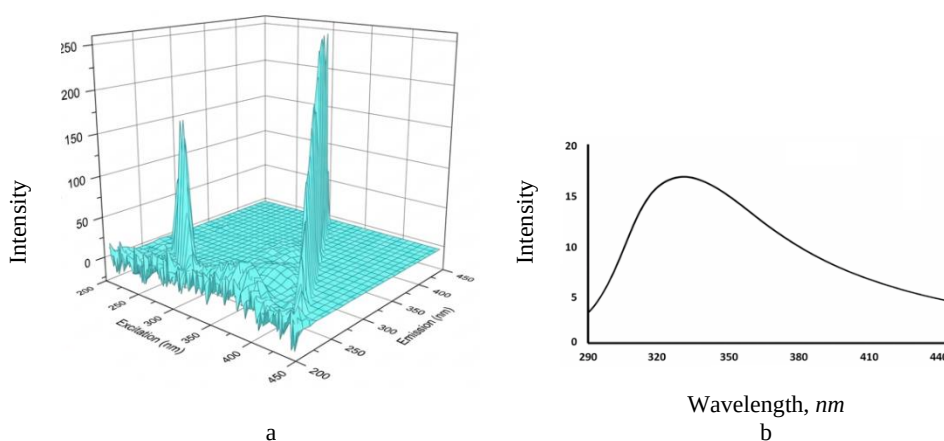


Fig. 3. 3D-EEM fluorescence spectrum of ADA (a) and the extracted emission spectrum at excitation wavelength of 280 nm (b).

From the obtained 3D-EEM spectra of ADA, D011_186 and their mixture, the emission spectra for the excitation at 280 nm, specific for protein tryptophan residues, were extracted and shown in Figs. 3, b; 4, b and 5, b, respectively.

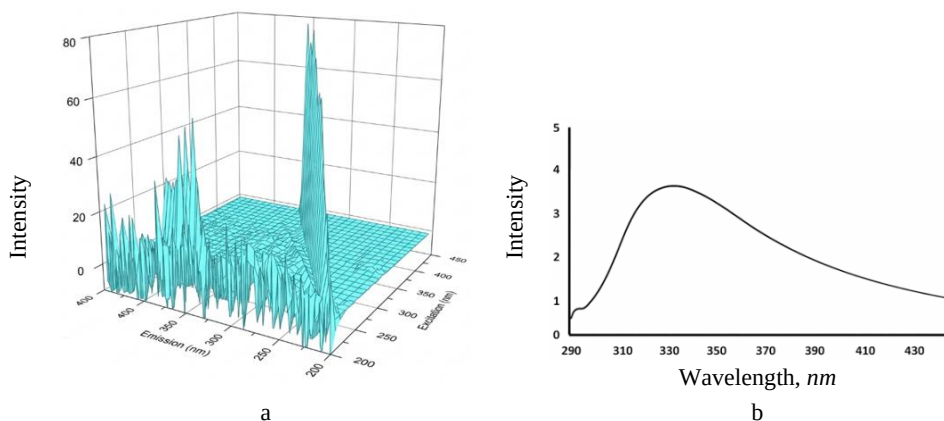


Fig. 4. 3D-EEM fluorescence spectrum of D011_186 (a) and the extracted-emission spectrum at excitation wavelength of 280 nm (b).

All these spectra are provided with the emission maxima around wavelength 340 nm of different intensity. The intensity of fluorescence is higher for ADA (Fig. 3, a) than for compound D011_186 (Fig. 4, b), and the intensity of fluorescence spectrum of their mixture (Fig. 5, b) represents a simple sum of the intensities of individual spectra.

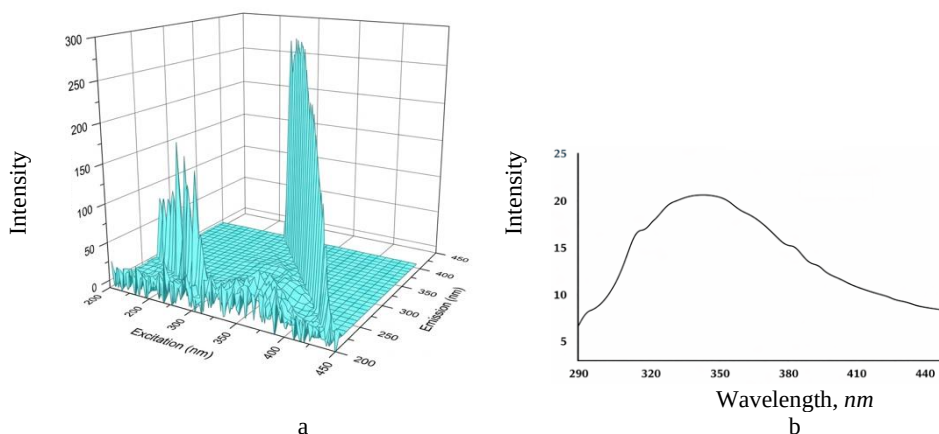


Fig. 5. 3D-EEM fluorescence spectrum of ADA + D011_186 mixture (a) and extracted emission spectrum at excitation wavelength of 280 nm (b).

The identical result was obtained at registering 2D fluorescence spectra: if the fluorescence of these samples were excited by wavelength of 280 nm, the emission maxima were registered in the region 330–350 nm.

Therefore, we implemented the recording of more complex, wide-range 3D EEM spectra hoping to detect a change in the orbital structures of optical electrons of the molecules bound to each other, which would be reflected in any emission/excitation of the ADA-D011_186 mixture that is absent from their individual spectra.

However, similar to the optical absorbance analysis, in this study we also failed to register any difference between the spectra of ADA+D011_186 mixture and the sum of individual ADA and D011_186 spectra.

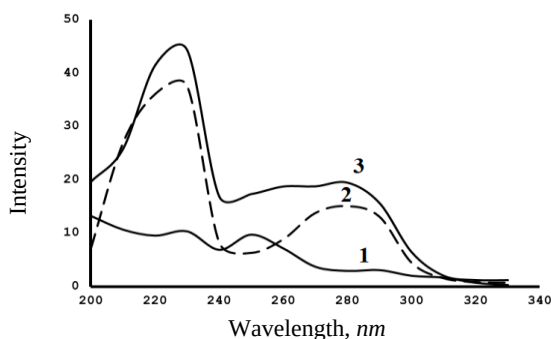


Fig. 6. 2D fluorescence excitation spectra of D011_186 (1); ADA (2) and their mixture (3) for emission maxima at wavelength of 340 nm.

This “bad” result is confirmed by the excitation spectra of emission at 340 nm, extracted from the 3D-EEM fluorescence spectra of all three studied samples (Fig. 6).

The fluorescence intensity of D011_186 is relatively low suggesting that it has weak intrinsic fluorescence under these excitation/emission conditions.

ADA shows essentially higher fluorescence intensity, associated with a higher exposure or greater contribution of fluorescent amino acid residues such as tryptophan. The ADA-D011_186 mixture exhibits the highest fluorescence intensity among the three systems, which, however, is a simple sum of intensities of two interacting subjects, ADA and D011_186.

Results and Discussion. In fact, the described in the present work investigation of the ADA interaction with the compound, possessing essential inhibitory ability, failed to register any spectral reflectance of the interaction. The interaction ADA-D011_186 did not disturb the electron orbitals in either of the two compounds, which would be reflected by a change(s) in the optical absorption and/or fluorescence spectra of their mixture compared to the simple sum of their individual spectra.

There are a number of studies devoted to the role of Trp residues in the stabilisation of various states of ADA and in realisation of enzymatic function, namely the deamination of adenosine. Kurtz et al. reported that the complex of ADA with substrate analogues resulted in resonant energy transfer from Trp to the ligand, indicating the proximity of the Trp residue in ADA to the substrate binding site [14]. Similarly, based on the decrease in Trp fluorescence in calf thymus ADA after binding of inhibitor analogues, Phillips et al. concluded that although the Trp does not coordinate directly with the substrate, it may be located near the active site of the enzyme [15].

Conclusion. The compound D011_186 exhibited potent ADA inhibition activity with an IC_{50} value of $40 \pm 1.1 \mu g/mL$. To reveal the mechanism of the observed inhibition, the interaction between the compound and ADA was investigated by comparison of their individual optical absorbance and fluorescence spectra with those of their mixture. However, no change was found at least in the region of tryptophan residues in ADA. Hence, the inhibitory effect of the compound tested is not accompanied by distortion of the orbitals of optical electrons of the interacting compounds, particularly, near the Trp residues in ADA molecule.

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REFERENCES

1. Gao Z., Wang X., et al. The roles of Adenosine Deaminase in Autoimmune Diseases. *Autoimmun. Rev.* **20** (2021), 102709.
<https://doi.org/10.1016/j.autrev.2020.102709>
2. Van der Weyden M.B., Kelley W.N. Human adenosine deaminase. Distribution and properties. *Journal of Biological Chemistry* **251** (1976), 5448–5456.
[https://doi.org/10.1016/S0021-9258\(17\)33080-6](https://doi.org/10.1016/S0021-9258(17)33080-6)
3. Blackburn M.R., Kellems R.E. Adenosine Deaminase Deficiency: Metabolic Basis of Immune Deficiency and Pulmonary Inflammation. In F.W. Alt (ed.). *Adv. Immunol.* **86** (2005), 1–41.
[https://doi.org/10.1016/S0065-2776\(04\)86001-2](https://doi.org/10.1016/S0065-2776(04)86001-2)

4. Banales J.L., Pineda P.R., et al. Adenosine Deaminase in the Diagnosis of Tuberculous Pleural Effusions. A Report of 218 Patients and Review of the Literature. *Chest* **99** (1991), 355–357.
<https://doi.org/10.1378/chest.99.2.355>
5. Zhang Y.-Z., Zhou B., et al. Fluorescence Study on the Interaction of Bovine Serum Albumin with p-Aminoazobenzene. *J. Fluoresc.* **18** (2008), 109–118.
<https://doi.org/10.1007/s10895-007-0247-4>
6. Punith R., Hegde A. H., Jaldappagari S. J. Binding of an Anti-inflammatory Drug Lornoxicam with Blood Proteins: Insights from Spectroscopic Investigations. *Fluoresc.* **21** (2011), 487–495.
<https://doi.org/10.1007/s10895-010-0735-9>
7. Zhang H., Gao S., Xiong Z., Liu S. Fluorometric Probing on the Binding of Hematoxylin to Serum Albumin. *Mol. Biol. Rep.* **36** (2009), 2299–2306.
<https://doi.org/10.1007/s11033-009-9448-z>
8. Grigoryan K.R., Shilajyan H.A. Fluorescence 2D and 3D Spectra Analysis of Tryptophan, Tyrosine and Phenylalanine. *Proceedings of the YSU B. Chem. and Biol. Sci.* **51** (2017), 3–7.
<https://doi.org/10.46991/PYSUB.2017.51.1.003>
9. Mocz G., Ross J.A. Fluorescence Techniques in Analysis of Protein-Ligand Interactions. In: M.A. Williams, T. Daviter (eds.). *Protein-Ligand Interactions. Methods in Molecular Biology*, NJ, Totowa, Humana Press **1008** (2013), 169–210.
https://doi.org/10.1007/978-1-62703-398-5_7
10. Sharoyan S.G., Antonyan A.A., Mardanyan S.S. Isolation, Purification and Comparative Investigation of Adenosine Deaminase from Five Parts of Cattle Brain. *Biochemistry (Moscow)* **59** (1994), 239–245.
<https://api.semanticscholar.org/CorpusID:88790705>
11. Mardanyan S., Sharoyan S., et al. Tryptophan Environment in Adenosine Deaminase: Enzyme Modification with N-bromosuccinimide in the Presence of Adenosine and EHNA Analogs. *Biochem. Biophys. Acta* **1546** (2001), 185–195.
[https://doi.org/10.1016/s0167-4838\(01\)00141-8](https://doi.org/10.1016/s0167-4838(01)00141-8)
12. Rawat B., Shukla S., et al. Synthesis Characterization and Anti-Inflammatory Activities of Substituted Aniline Oxadiazolyl Derivatives. *International Journal of Scientific Research in Science, Engineering and Technology* **3** (2017), 291–295.
<https://ijsrset.com/IJSRSET173467>
13. Bakaryan A., Karapetyan L., et al. Adenosine Deaminase – A Target for New Piperazine Derivatives. *Biophys. Chem.* **277** (2021), 106658.
<https://doi.org/10.1016/j.bpc.2021.106658>
14. Kurz L.C., LaZard D., Frieden C. Protein Structural Changes Accompanying Formation of Enzymatic Transition States: Tryptophan Environment in Ground-State and Transition-State Analogue Complexes of Adenosine Deaminase. *Biochemistry* **24** (1985), 1342–1346.
<https://doi.org/10.1021/bi00327a011>
15. Philips A., Robbins D., Coleman M., Barkley M. Immunoaffinity Purification and Fluorescence Studies of Human Adenosine Deaminase. *Biochemistry* **26** (1987), 2893–2903.
<https://doi.org/10.1021/bi00384a034>

Ա. Կ. ԲԱԿԱՐՅԱՆ

ԱԴԵՆՈԶԻՆԴԵԱՄԻՆԱԶԻ ԵՎ D011_186 ՄԻԱՑՈՒԹՅԱՆ
ՓՈԽԱԴՐՈՒԹՅԱՆ ՀԵՏԱԶՈՏՈՒՄԸ ՖԼՅՈՒՈՐԵՍԵՆՑԻԱՅԻ ԵՎ
ՈՒՄ-ՏԵՍ ՍՊԵԿՏՐՈՍԿՈՊԻԱԿԱՆ ՄԵԹՈԴՆԵՐՈՎ

Ադենոզինդեամինազը (ԱԴԱ) կաթնասունների պուրինային նյութափոխանակության և իմունային համակարգի կարևոր ֆերմենտ է: Դրա ակտիվությունը աճում է պաթոլոգիաներում բերելով հակաբորբոքային ադենոզինի կոնցենտրացիայի նվազմանը, ինչը սրում է բորբոքումը: Հետևաբար, ԱԴԱ արգելակիչների հայտնաբերումն ու զարգացումը կենսաքիմիական և դեղաբանական կարևոր խնդիրներ են: Մենք փորձարկել ենք նոր սինթեզված անիլինի

և պիպերազինի մոտավորապես 100 ածանցյալներ դիտարկելով դրանց ԱԴԱ-ի ակտիվության վրա ունեցած ազդեցությունը: Տվյալ ուսումնասիրությունը նկարագրում է ԱԴԱ-ի ակտիվության վրա անիլինի ածանցյալներից մեկի՝ D011_186-ի արգելակիչ ազդեցությունը: Այն ցուցաբերել է բավականին բարձր արգելակիչ ակտիվություն՝ IC_{50} -ը $40 \pm 1,1$ մկգ/մլ արժեքով: ԱԴԱ-ի և D011_186-ի միջև փոխազդեցությունը ուսումնասիրվել է ՈՒՄ-Տես և ֆլուորեսցենտային սպեկտրոսկոպիայի մեթոդներով: Դիֆերենցիալ ՈՒՄ-Տես սպեկտրոսկոպիան ԱԴԱ-ի և միացություն խառնուրդի օպտիկական կլանման և նրանց անհատական սպեկտրների գումարի միջև էական տարբերություն չի բացահայտել: Այնուհետև համեմատվել են ԱԴԱ-ի և միացություն անհատական 2D և 3D ֆլուորեսցենտային սպեկտրները դրանց խառնուրդի սպեկտրների հետ: Սակայն, փոփոխություններ, որոնք ցույց կտային դրանց փոխազդեցությունը, չեն հայտնաբերվել: Այսպիսով, չնայած D011_186 միացությունը արգելակում է ԱԴԱ-ի ակտիվությունը, այն չի ուղեկցվում դրանց օպտիկական էլեկտրոնների օրբիտալների փոփոխությամբ:

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АНАЛИЗ ВЗАИМОДЕЙСТВИЯ АДЕНОЗИНДЕАМИНАЗЫ И СОЕДИНЕНИЯ D011_186 МЕТОДАМИ ОПТИЧЕСКОЙ И ФЛУОРЕСЦЕНТНОЙ СПЕКТРОСКОПИИ

Аденозиндезаминаза (АДА) – фермент, играющий важную роль в метаболизме пуринов у млекопитающих и в функционировании иммунной системы. При патологических состояниях активность АДА возрастает, что приводит к снижению концентрации противовоспалительного агента аденозина и, как следствие, к усилению воспаления. В связи с этим поиск и разработка ингибиторов АДА являются актуальной биохимической и фармакологической задачей. Мы исследовали способность ингибировать АДА у примерно 100 новых синтезированных производных анилина и пиперазина. В настоящем исследовании описано ингибирование АДА одним из протестированных производных анилина – соединением D011_186. Данное соединение продемонстрировало высокую ингибирующую активность со значением IC_{50} , равным $40 \pm 1,1$ мкг/мл. Взаимодействие между АДА и D011_186 изучали методами УФ-ВИД и флуоресцентной спектроскопии. Анализ взаимодействия АДА с соединением методом дифференциальной УФ-ВИД спектроскопии не выявил существенных различий между оптическим поглощением их смеси и суммой спектров отдельных компонентов. Затем, спектры двумерной (2D) и трехмерной (3D) флуоресцентной спектроскопии АДА и соединения по отдельности сравнивали со спектрами их смеси. В этих спектрах флуоресценции также не было обнаружено изменений, указывающих на взаимодействие исследуемых компонентов. Таким образом, несмотря на то, что соединение D011_186 эффективно ингибирует активность АДА, это ингибирование не сопровождается изменением состояния орбиталей оптических электронов взаимодействующих компонентов.